Video Article

The Tail Suspension Test

Adem Can[1,*], David T. Dao[1,2,*], Chantelle E. Terrillion[3], Sean C. Piantadosi[1], Shambhu Bhat[1], Todd D. Gould[1,3,4]

[1]Department of Psychiatry, University of Maryland School of Medicine
[2]Tulane University School of Medicine
[3]The Program in Neuroscience, University of Maryland
[4]Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine

[*]These authors contributed equally

Correspondence to: Todd D. Gould at gouldlab@me.com

URL: http://www.jove.com/video/3769/
DOI: 10.3791/3769

Keywords: Neuroscience, Issue 59, animal models, behavioral analysis, neuroscience, neurobiology, mood disorder, depression, mood stabilizer, antidepressant,

Date Published: 1/28/2012

Citation: Can, A., Dao, D.T., Terrillion, C.E., Piantadosi, S.C., Bhat, S., Gould, T.D. The Tail Suspension Test. J. Vis. Exp. (59), e3769, DOI : 10.3791/3769 (2012).

Abstract

The tail-suspension test is a mouse behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors. Mice are suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. During this test, typically six minutes in duration, the resulting escape oriented behaviors are quantified. The tail-suspension test is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant compounds. Here, we describe the details required for implementation of this test with additional emphasis on potential problems that may occur and how to avoid them. We also offer a solution to the tail climbing behavior, a common problem that renders this test useless in some mouse strains, such as the widely used C57BL/6. Specifically, we prevent tail climbing behaviors by passing mouse tails through a small plastic cylinder prior to suspension. Finally, we detail how to manually score the behaviors that are manifested in this test.

Video Link

The video component of this article can be found at http://www.jove.com/video/3769/

Protocol

1. Materials

1. Suspension Box

The tail-suspension test (TST) involves suspending mice above the ground by their tails. At the most basic level, the procedure only requires a suspension bar or shelf ledge, and tape. However, the experimenter should consider the use of a background that provides optimal contrast. Additionally, it is prudent to take steps to prevent mice from observing other animals that are being tested. In our laboratory, we use specially manufactured tail suspension boxes (Four-Hour Day, Baltimore MD), made of plastic with the dimensions (55 height X 60 width X 11.5 cm depth). In order to prevent animals from observing or interacting each other, each mouse is suspended within its own three-walled rectangular compartment (55 height X 15 width X 11.5 cm depth). The mouse is suspended in the middle of this compartment and the width and depth are sufficiently sized so that the mouse cannot make contact with the walls. In this setting, the approximate distance between the mouse's nose and the apparatus floor is 20-25 cm. There are four such identical compartments in the apparatus allowing us to test four mice at a time. An aluminum suspension bar (1 cm. height X 1 cm. width x 60 cm. length), used to suspend the tail of each mouse, is positioned on the top of the box. The dimensions that we use in our laboratory should be regarded as a general reference. For example, the size of individual compartments of the tail-suspension box can be increased if a large outbred mouse strain (e.g. CD-1) is used.

At the bottom of each compartment we place a detachable aluminum tray that collects feces or urine from the animals. We use a dark grey box for albino animals and a cream colored box for mice of other coat colors. This arrangement gives us better contrast and therefore more reliable behavioral scoring of the test.

2. Tape

Tape should adhere securely to both the mouse's tail and the suspension bar, and be strong enough to carry the weight of the mouse being tested (e.g. TimeMed Labeling Systems, Inc, Burr Ridge IL, 1.9 cm width). However, the tape should not be excessively sticky since at the end of the session it will be removed from the tail. We cut 17 cm fragments of tape and place a mark 2 cm from one end. This 2 cm portion was used for attaching the tape to the tail, while the remaining 15 cm was used for suspension of the mouse.

3. Timer

4. Video recording device

A video camera and a tripod (or other support structure) are needed. Since this test usually involves multiple animals being tested at the same time, live scoring will be difficult and is not advised. The video camera should record in high enough resolution to render a quality picture that will be used later for behavioral scoring. Always make certain that there is sufficient recording memory in the camera before starting the test. We use a video camera that records digitally without the use of physical media (i.e. video cassette), allowing for straightforward transfer of videos.

5. White noise generator

The noise generator should mask intermittent environmental sounds. The use of a noise generator is particularly recommended in laboratory environments where sudden loud noises can be heard that would potentially startle mice. In our experimental room the ambient noise level (without the white noise generator activated) is around 60 dB. The total sound level with the white noise generator activated at the location where the TST is conducted is 70-72 dB. However, it should be noted that these figures are provided as example only, and each laboratory should select the right noise levels according to their unique environment and circumstances.

6. Cleaning supplies

The suspension box should be wiped thoroughly after each session with a sterilization solution (e.g. MB-10, Quip Laboratories Inc., Wilmington, DE, or similar).

7. Climbstoppers (optional: dependent upon strain used)

Mice of some backgrounds, such as C57BL/6, may climb their tails during the test[1]. Clear hollow cylinders (4 cm length, 1.6 cm outside diameter, 1.3 cm inside diameter, 1.5 grams) that are cut to four cm length (by FourHourDay Inc, Baltimore MD) from polycarbonate tubing (#8585K41, McMaster-Carr, Santa Fe Springs, CA) are placed around the tails of mice to prevent such tail climbing behavior[2,3]. These devices can be made in any laboratory with common tools and materials.

2. Behavioral Procedures

The overall experimental design should reflect proper counterbalancing between variables specific to your experiment. For example, in our experiments we try to represent each group equally in every TST session (i.e., if there are four treatment groups, each will be represented in each session). Also, the positions of mice are rotated in a way such that mice from each treatment group are placed in a different position in each session.

1. Place the camera in position. The camera should be as close as possible in order to obtain the highest possible resolution of the animals.
2. Tape fragments that will be used during the session should be cut, marked, and prepared for the session.
3. Start the white noise generator, if being used, before the mice are introduced to the testing room. The level of white noise should only be enough to mask external noises. Avoid a high volume and make sure the same level of white noise is used for all animals.
4. Bring the animals into the testing room. If the room where the animals reside and the testing room are adjacent to each other with similar ambient conditions, no acclimation period may be necessary. Otherwise, place the animals in the testing room for a period of acclimation (generally at least one hour). Be aware that the other animals placed in the same room can sense olfactory and ultrasonic cues.
5. If a strain known to climb their tails, such as C57BL6/J, is being used place Climbstoppers around the tails prior to applying the tape.
6. Adhere the tape to the tails of mice. Use the marked end of the tape and make sure the tape sticks to the tail and back on itself. The tape should be applied to the very end of the tail with 2-3 millimeters of tail remaining outside of the tape.
7. Once each piece of tape is attached to a mouse tail stick the middle portion of the tape to the inner walls of the cage. This will help prevent tangling of tape pieces to each other. Note that confinement in this fashion may cause stress and that it is important to complete the process of applying the tape to each animal as soon as possible.
8. Once all tape is applied, start the recording and identify the session before the mice are suspended.
9. Suspend the animals by placing the free end of the tape on the suspension bar or shelf in an order that is counterbalanced between treatment groups. Suspend mice in a way that is does not obstruct the camera view because the entire TST session will be scored and obstructing the camera will lead to the inability to assess behaviors during that time. Check that all tape ends are hanging parallel to the suspension bar or shelf with a similar length of slack for each mouse.
10. At the end of the session (which is typically six minutes), return the animals to their homecage and carefully remove the tape from each tail by gently pulling it off. Do not rip the tape from the tail since doing so may cause pain to the mice.
11. After returning the mice to their colony room discard the fecal boli and urine from the collection trays and wipe the apparatus with a sterilizing solution.

3. Behavior Analysis

1. Generally, the TST is from start to finish six minutes in length. Unlike another widely used antidepressant efficacy procedure, the forced swim test, the whole session is scored. This is due to the general finding that mice tend to manifest immobility earlier in the TST.
2. In our laboratory we upload the video files directly from the camera to a PC where the analysis is conducted.
3. During the behavioral analysis the time that each mouse spends as mobile is measured. While it is possible to measure the immobility time directly, we have found it easier to detect and measure active movements rather than the lack of such movements.
4. The most important aspect of TST behavioral analysis is the consistent identification of movements that are recorded as bona fide mobility. Mice, especially at the beginning of the session, manifest behaviors that are overtly escape related. These include trying to reach the walls of the apparatus and the suspension bar, strong shaking of the body, and movement of the limbs akin to running. These movements clearly constitute mobility. These behaviors then subside and become subtler. In our laboratory small movements that are confined to the front legs but without the involvement of the hind legs are not counted as mobility. Additionally, oscillations and pendulum like swings that are due to the momentum gained during the earlier mobility bouts also are not counted as mobility.
5. In our laboratory we use an on-screen stopwatch software for time measurements (Xnote Stopwatch, dnSoft Research Group). Two separate stopwatches are used on the screen. The first stopwatch counts down from 360 seconds and alerts the observer when the behavioral analysis period ends. The second stopwatch —controlled by the observer—measures the time spent mobile. Certain stopwatch software has the ability to assign keys to start and stop functions, so that on-screen the keyboard can control the stopwatch. Instead of a regular keyboard, we use an input device commonly known as a 'gamepad' to control the stopwatches.

6. If there is more than one mouse tested and present on the screen it is a good idea to cover the other animals so that their movements will not distract the observer. This can be accomplished by using another program window or by physically covering the other mice on the screen with paper.
7. There may be some bias occurring if the total amount of mobility time elapsed for a particular mouse is visible prior to completion of the analysis session. If an on-screen stopwatch is used, we suggest to cover all but the millisecond decimals of the stopwatch. By covering the stopwatch the observer only knows whether the stopwatch is on or off at any point but does not know the total time elapsed and therefore cannot be affected by any bias. Using this approach the observer, while blind to the group assignments of the animals, will not have a general idea of level of mobility in each animal.
8. An interobserver reliability test should be conducted for every new observer before beginning to collect data from test animals. In our laboratory each new observer first watches an experienced observer while he or she is scoring. After the new observers gain enough confidence to differentiate mobility from immobility, they then score in real time with the experienced observer watching and pointing out any mistakes. Once this phase is successfully completed, the new observers will analyze a specific set of TST videos that we maintain in our laboratory for training purposes. Only after a high level of interobserver correlation is obtained with the experienced observer does an investigator analyze TST videos from actual experiments. We archive the data from these training analyses to constitute an internal standard for the laboratory. We have observed differences between strains in the manner in which they express mobility (and immobility) behaviors, and mean immobility time between sexes. When a new strain, sex, or genetically modified mouse model is tested in the laboratory it is necessary to again undertake this type of reliability analysis.

4. Representative Results

We have used the TST to assess antidepressant-like responses to lithium treatment in various mouse strains (Figure 1)[3]. Experimental details of this experiment are published in Can *et al.*, 2011[3].

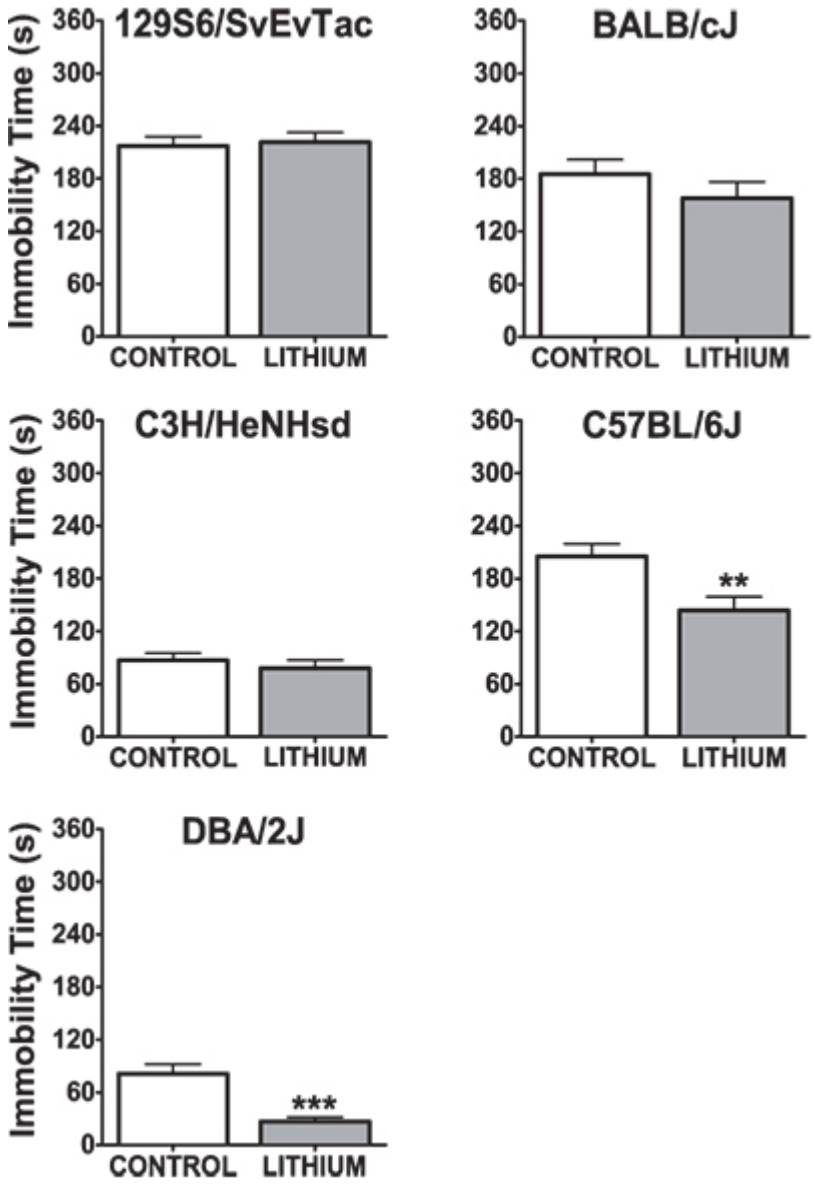

Figure 1. Immobility time in the tail suspension test after chronic lithium administration in five inbred mouse strains. Mice were group housed, four mice per cage. Mice in the lithium treatment group received lithium chow containing 4 g/kg LiCl for three weeks. Control animals received identical food without the lithium chloride. **:p<0.01, ***:p<0.001 denote a significant, unpaired t-test. Data are expressed as mean ± SEM. n:10-12 animals per group for each strain. (figure reproduced from [3]).

In this example, a significant treatment effect of chronic lithium delivered in food is observed only in C57BL/6J and DBA/2J strains. In the other three strains, no statistically significant decrease in immobility time was observed. These data indicate that an antidepressant-like response to chronic lithium treatment is strain dependent as has also been observed with other antidepressant medications[4-8].

Discussion

Development of the TST by Steru *et al.*[9] was influenced by the previously developed forced swim test[10-12]. Similar to the forced swim test, in the TST mice are placed in an inescapable but moderately stressful situation. Lack of escape related behavior is considered immobility. Like the forced swim test, the TST is a test best validated for the evaluation of antidepressant efficacy of drugs, but also used to evaluate the effects of environmental, neurobiological, and genetic manipulations[13-18]. In contrast to the forced swim test, in the TST there is no risk of hypothermia due to submersion in water[19]. The experimenters should keep in mind that while the forced swim test and TST are similar *prima facie*, there are various important differences in their sensitivity and performance and the results of one test may not necessarily be replicated in the other. For an excellent description of the differences between these two tests see the following references[4,20,21]. Though most antidepressants take weeks to exert clinically significant effects in patients, antidepressants can exert their effects in the TST and forced swim test both following acute and chronic treatments[20,22,23]. However, there are many other behavioral tests used to assess antidepressant-like effects in mice that are generally sensitive to chronic treatment only. These include chronic unpredictable stress[24], social defeat stress[25], and novelty suppressed feeding[26].

In addition to its small footprint, low cost and relative ease of setup, automation of data collection in the TST is also possible[9,27,28]. Two main approaches for automation are electro-mechanical measurement systems and video analysis. In the electro-mechanical approach, the animal is suspended from a strain gauge and the movements of the animals are measured (e.g. Med Associates Inc, St. Albans, VT; Harvard Apparatus, Holliston, MA). Another approach is software-based analysis of TST video recordings (e.g. Noldus Inc., Netherlands; Cleversys Inc., Reston, VA). However, even with automation, human intervention will still be necessary, since the applied parameters must be adjusted for each new mouse model or strain and the results should be verified by a human observer for quality control purposes.

One persistent problem that deserves special attention with the TST is the tail-climbing behavior of some mice, especially the commonly used C57BL/6 strain[1,20]. These animals have a propensity toward reaching for and climbing their own tails. Mice that successfully climb their tails have learned that escape is possible. Such mice should therefore be excluded from the analysis, which increases the number of mice required and decreases reliability of the procedure. The fact that the C57BL/6 mouse strain is the most common strain used in neurobiological and genetic research exacerbates the influence of this problem. In this paper, we detailed a solution to tail-climbing behavior that was developed in our laboratory[2,3]. In order to prevent the tail-climbing behavior, we place hollow cylinders around base of the tail of mice. The mice cannot hold onto these cylinders and therefore cannot climb their tails. We have observed that no mice have climbed their tails when using this approach[2,3]. Additionally, we have shown that the antidepressant-like effects of lithium, reported previously in the TST without these types of cylinders[29], are still present when using them[3].

It should be noted that the TST is not recommended for heavier rodents such as rats, since it is potentially painful for these animals to support their weight only by their tails. By the same token, caution should be taken with unusually heavy mice (e.g. mice used for modeling obesity), and in these cases the experimenters should look for alternative tests such as the forced swim test[10] It should be kept in mind that any manipulations that may affect the overall activity levels may potentially alter mobility in the TST leading to spurious conclusions. Because of this, it is important to verify the results of TST with separate behavioral tests that measure overall activity levels in mice such as the open-field test [30].

Disclosures

Authors declare no conflicts of interest.

Acknowledgements

This study has been supported by the grant NIHM R01 MH091816 and R21 MH084043 to TDG.

References

1. Mayorga, A.J. & Lucki, I. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl).* **155**, 110-112 (2001).
2. Dao, D.T., *et al.* Mood Disorder Susceptibility Gene CACNA1C Modifies Mood-Related Behaviors in Mice and Interacts with Sex to Influence Behavior in Mice and Diagnosis in Humans. *Biological Psychiatry.* **68**, 801-810 (2010).
3. Can, A.L., *et al.* Antidepressant-like responses to lithium in genetically diverse mouse strains. *Genes, Brain and Behavior.* **10**, 434-443 (2011).
4. Bai, F., Li, X., Clay, M., Lindstrom, T., & Skolnick, P. Intra- and interstrain differences in models of "behavioral despair". *Pharmacol. Biochem. Behav.* **70**, 187-192 (2001).
5. Cervo, L., *et al.* Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. *J. Neurosci.* **25**, 8165-8172 (2005).
6. David, D.J., Renard, C.E., Jolliet, P., Hascoet, M., & Bourin, M. Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology (Berl).* **166**, 373-382 (2003).
7. Dulawa, S.C., Holick, K.A., Gundersen, B., & Hen, R. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology.* **29**, 1321-1330 (2004).
8. Lucki, I., Dalvi, A., & Mayorga, A.J. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl).* **155**, 315-322 (2001).
9. Steru, L., Chermat, R., Thierry, B., & Simon, P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl).* **85**, 367-370 (1985).
10. Can, A., Dao, D. T., Arad, M., Terrillion, C. E., Piantadosi, S.C., & Gould, T.D. The Mouse Forced Swim Test. *J. Vis. Exp.* (58), e3638, DOI: 10.3791/3638 (2012).
11. Porsolt, R.D., Bertin, A., & Jalfre, M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **229**, 327-336 (1977).

12. Porsolt, R.D., Anton, G., Blavet, N., & Jalfre, M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* **47**, 379-391 (1978).
13. Crowley, J.J., Blendy, J.A., & Lucki, I. Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology (Berl).* **183**, 257-264 (2005).
14. Karolewicz, B. & Paul, I.A. Group housing of mice increases immobility and antidepressant sensitivity in the forced swim and tail suspension tests. *European Journal of Pharmacology.* **415**, 197-201, doi:10.1016/s0014-2999(01)00830-5 (2001).
15. Lad, H.V., Liu, L., Paya-Cano, J.L., Fernandes, C., & Schalkwyk, L.C. Quantitative traits for the tail suspension test: automation, optimization, and BXD RI mapping. *Mammalian. Genome.* **18**, 482-491, doi:10.1007/s00335-007-9029-1 (2007).
16. Liu, X. & Gershenfeld, H.K. Genetic differences in the tail-suspension test and its relationship to imipramine response among 11 inbred strains of mice. *Biol. Psychiatry.* **49**, 575-581 (2001).
17. Ripoll, N., David, D.J., Dailly, E., Hascoet, M., & Bourin, M. Antidepressant-like effects in various mice strains in the tail suspension test. *Behav. Brain. Res.* **143**, 193-200 (2003).
18. Mineur, Y.S., Belzung, C., & Crusio, W.E. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behav. Brain. Res.* **175**, 43-50 (2006).
19. Thierry, B., Stéru, L., Simon, P., & Porsolt, R.D. The tail suspension test: Ethical considerations. *Psychopharmacology.* **90**, 284-285, doi:10.1007/bf00181261 (1986).
20. Cryan, J.F., Mombereau, C., & Vassout, A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci. Biobehav. Rev.* **29**, 571-625 (2005).
21. O'Leary, O.F. & Cryan, J.F. In *Mood and Anxiety Related Phenotypes in Mice.* Gould, T.D., ed., Vol. 42,119-137, Humana Press, (2009).
22. Detke, M.J., Johnson, J., & Lucki, I. Acute and Chronic Antidepressant Drug Treatment in the Rat Forced Swimming Test Model of Depression. *Experimental and Clinical Psychopharmacology.* **5**, 107-112 (1997).
23. Rupniak, N.M.J. Animal models of depression: challenges from a drug development perspective. *Behavioural Pharmacology.* **14**, 385-390 (2003).
24. Strekalova, T. & Steinbusch, H. In *Mood and Anxiety related phenotypes in mice: Characterization using behavioral tests.* **42**, 153-176 (2009).
25. Bartolomucci, A., Fuchs, E., Koolhaas, J.M. & Ohl, F. In *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests.* **42**, 261-275 (2009).
26. Samuels, B.A. & Hen, R. In *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests.* **63**, 107-121 (2011).
27. Juszczak, G.R., *et al.* The usage of video analysis system for detection of immobility in the tail suspension test in mice. *Pharmacology Biochemistry and Behavior.* **85**, 332-338, doi:10.1016/j.pbb.2006.08.016 (2006).
28. Crowley, J.J., Jones, M.D., O'Leary, O.F., & Lucki, I. Automated tests for measuring the effects of antidepressants in mice. *Pharmacology Biochemistry and Behavior.* **78**, 269-274, doi:10.1016/j.pbb.2004.03.014 (2004).
29. Gould, T.D., *et al.* Involvement of AMPA receptors in the antidepressant-like effects of lithium in the mouse tail suspension test and forced swim test. *Neuropharmacology.* **54**, 577-587 (2008).
30. Gould, T.D., Dao, D.T., & Kovacsics, C.E. In *Mood and Anxiety Related Phenotypes in mice: characterization using behavioral tests.* Gould, T.D., ed., Vol. 42, Humana Press, (2009).

